

considered 100% growth and the growth of cells in wells with compounds is compared to this. KSP inhibitors inhibit cell proliferation in human ovarian tumor cell lines (SKOV-3).

[0421] A GI_{50} is calculated by plotting the concentration of compound in μM vs the percentage of cell growth of cell growth in treated wells. The GI_{50} calculated for the compounds is the estimated concentration at which growth is inhibited by 50% compared to control, i.e., the concentration at which:

$$100 \times [(Treated_{48} - T_0) / (Control_{48} - T_0)] = 50.$$

[0422] All concentrations of compounds are tested in duplicate and controls are averaged over 12 wells. A very similar 96-well plate layout and GI_{50} calculation scheme is used by the National Cancer Institute (see Monks, et al., J. Natl. Cancer Inst. 83:757-766 (1991)). However, the method by which the National Cancer Institute quantitates cell number does not use MTS, but instead employs alternative methods.

Example 6

[0423] Calculation of IC_{50} :

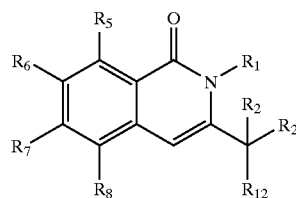
[0424] Measurement of a compound's IC_{50} for KSP activity uses an ATPase assay. The following solutions are used: Solution 1 consists of 3 mM phosphoenolpyruvate potassium salt (Sigma P-7127), 2 mM ATP (Sigma A-3377), 1 mM 1DTT (Sigma D-9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM $MgCl_2$ (VWR JT400301), and 1 mM EGTA (Sigma E3889). Solution 2 consists of 1 mM NADH (Sigma N8129), 0.2 mg/ml BSA (Sigma E7906), pyruvate kinase 7 U/ml, L-lactate dehydrogenase 10 U/ml (Sigma PO294), 100 nM KSP motor domain, 50 g/ml microtubules, 1 mM DTT (Sigma D9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM $MgCl_2$ (VWR JT4003-01), and 1 mM EGTA (Sigma E3889). Serial dilutions (8-12 two-fold dilutions) of the compounds are made in a 96-well microtiter plate (Corning Costar 3695) using Solution 1. Following serial dilution each well has 50 μl of Solution 1. The reaction is started by adding 50 μl of solution 2 to each well. This may be done with a multichannel pipettor either manually or with automated liquid handling devices. The microtiter plate is then transferred to a microplate absorbance reader and multiple absorbance readings at 340 nm are taken for each well in a kinetic mode. The observed rate of change, which is proportional to the ATPase rate, is then plotted as a function of the compound concentration. For a standard IC_{50} determination the data acquired is fit by the following four parameter equation using a nonlinear fitting program (e.g., Grafit 4):

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^s} + \text{Background}$$

[0425] where y is the observed rate and x the compound concentration.

What is claimed is:

1. A compound having the structure represented by Formula I:



(I)

wherein:

R_1 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, and optionally substituted heteroaralkyl-;

R_2 and $R_{2'}$ are independently chosen from hydrogen, optionally substituted alkyl-, optionally substituted alkoxy, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, and optionally substituted heteroaralkyl-; or R_2 and $R_{2'}$ taken together form an optionally substituted 3- to 7-membered ring;

R_{12} is selected from the group consisting of optionally substituted imidazolyl-, optionally substituted imidazolyl-, $-NHR_4$, $-N(R_4)(COR_3)$, $-N(R_4)(SO_2R_{3a})$; and $-N(R_4)(CH_2R_{3b})$;

R_3 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, $R_{15}O-$ and $R_{17}NH-$;

R_{3a} is chosen from optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, and $R_{17}NH-$;

R_{3b} is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, and optionally substituted heteroaralkyl-;

R_4 is chosen from hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heterocycl-, and optionally substituted heteroaralkyl-;

R_5 , R_6 , R_7 and R_8 are independently chosen from hydrogen, optionally substituted alkyl, optionally substituted alkoxy, halogen, hydroxyl, nitro, cyano, dialkylamino, alkylsulfonyl, alkylsulfonamido, alkylthio, carboxyalkyl, carboxamido, aminocarbonyl, optionally substituted aryl and optionally substituted heteroaryl;

R_{15} is optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted heteroaralkyl-; and

R_{17} is chosen from hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl;